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THE MANUFACTURE AND KEEPING QUALITY OF UNWASHED BUTTER.

By L. L. MULLER, B.Sc. (formerly Assistant Bacteriologist, Dairy Research Branch, Division of Dairying.)

SUMMARY.

Unwashed butter was compared in 51 commercial scale churnings at four butter factories with washed butter made from the same batches of cream. Grading and bacteriological and chemical analyses were performed on the butters when fresh, after cold storage in bulk for two months and four months, and after storage for two weeks and four weeks at 45–50°F. in pats both cut by hand from the bulk and prepared commercially by reworking under vacuum, followed by machine patting.

The results showed that, under Queensland conditions, the absence of the firming effect of the cold water used for washing the butter granules led to manufacturing difficulties unless the churning temperature was lowered. This prolonged the churning time and largely counterbalanced the saving in time through the omission of washing.

The manufacturing difficulties in some cases were reflected in the keeping quality of the unwashed butter in bulk or as hand-cut pats. However, where the manufacture of both control and experimental churnings was satisfactory, no significant difference in the keeping quality of washed and unwashed butter was observed.

In the case of vacuum-treated pats held at 45–50°F., a serious typical flavour defect developed in the unwashed butter but not in the washed control in 8 cases of 26. Although the flavour defect was described as "condensed-oxidised" it was not related to oxidation of the butterfat as judged by ferric thiocyanate estimation of peroxide values. No evidence of a bacteriological origin for the defect was found in the bacteriological examinations or in estimations of the non-protein nitrogen as a percentage of the total nitrogen. The cause of the defect therefore remains unexplained.

Advantages of the process were seen in the average increase of 0.43% found in the curd content of the unwashed butter and in a marked antioxidant effect which was observed and ascribed to the extra curd.

Estimations of non-protein nitrogen/total nitrogen ratios showed that the percentage of non-protein nitrogen tended to be higher in the unwashed butter under Queensland conditions. This may have been related to the quality of the original cream.

Due to the manufacturing difficulties encountered and the consequent variable opportunities for bacterial multiplication before examination, no conclusion could be reached on the effect of non-washing on the bacteriological quality of the resultant butter.

It was concluded that no recommendation should be made for the widespread adoption of non-washing during butter manufacture but that the process could prove very useful in cases of difficulty with the water supply or refrigeration at particular factories.

I. INTRODUCTION.

The washing of the butter granules with chilled water has always been regarded as an important step in the manufacture of butter of good keeping quality. However, there was little evidence to support this belief, and in view of the economic advantages which would result a number of investigators have recently examined the effect of omitting washing with water from the manufacturing procedure. The Danish State Dairy Experiment Station (1939) reported that unwashed butter had been found equal in quality to the normal washed butter. Jensen, Wiley, Trembath and Itzerott (Jensen 1942) found that the curd content of butter could be increased by not washing and that the keeping quality was not affected. Pont (1946) found some tendency for unwashed butter to have inferior keeping quality, but in later experiments (Pont 1948) this inferiority was not apparent.

The experiments at this stage were somewhat indecisive and did not give information on aspects such as manufacturing procedure. It was therefore decided to conduct experiments to determine the suitability of unwashed butter under Queensland conditions and to supplement the information available.

The summarized results of these experiments were conveyed to the dairy industry in Queensland by Nichols (1952). Since then Arnott, Greene and Gibson (1952) in Canada and McDowall, Singleton and O'Dea (1953) in New Zealand have published the results of similar experiments.

II. PROCEDURE.

The experiments were conducted over the period July 1949 to May 1951 at four commercial butter factories. The cream in each case was graded choice, cream acidity being in the range of 0·24–0·47% lactic acid. The first half of the vat of pasteurized cream was churned by the normal method, the remaining cream being reduced in temperature and held at the lower temperature until the same churn was again available for the manufacture of the unwashed butter. Holding vat rinsing with water was avoided, no breakwater was used during churning and buttermilk was used for all additions to the unwashed butter for moisture control purposes. Manufacturing details were recorded for each batch of churnings, with particular reference to the timing of the operations up to the completion of the washing stage.

Two boxes of each churning were held in cold storage (about 10°F.); one box was graded after two months and the other after four months. A separate box was used for grading, chemical and bacteriological analysis of the fresh butter.

In the early stages of the experiment arrangements were made for the bulk of each churning to be made into pats by the Queensland Butter Marketing Board, the process involving re-working in an Abel vacuum machine followed by machine patting. Sample pats were then held at 45–50°F. for grading and bacteriological and chemical analysis at two weeks and four weeks. However, defects occurred as discussed below which necessitated the discontinuance of vacuum treatment and machine patting. Pats were then cut manually from the box which was used for the initial grading and these were stored and examined as above. To further investigate the occurrence of defects in vacuum pats, three boxes of each of the last 22 pairs of churnings were treated by the Butter Marketing Board and sample pats were held at 45–50°F. for comparison after two and four weeks with the pats cut by hand.

III. ANALYTICAL METHODS.

Analyses of the butter for moisture, salt, curd and fat content were performed by the Kohman (1919) method. Bacteriological analyses and pH estimations were carried out on duplicate samples by the methods specified by Muller and Nichols (1950). Microscopic examination of the butter to obtain a count of the moisture droplets over 30μ in diameter was carried out by the method of Muller (1952). Peroxide values were determined by the ferric thiocyanate method of Loftus Hills and Thiel (1946), using as solvent a mixture of 70% chloroform and 30% methanol.

Where determinations of non-protein-nitrogen/total nitrogen ratios in the butter serum were made, the fat-free serum, obtained by centrifuging a 250 ml. centrifuge bottle filled with butter, decanting the fat and extracting the remaining fat with petrol ether, was weighed and made up to 50 ml. with distilled water. Total nitrogen values were determined by the Kjeldahl method on an aliquot of the diluted serum. Some determinations of non-protein nitrogen were made by the Kjeldahl method on the filtrate after precipitation by making 40 ml. of the diluted serum up to 100 ml. with 15% trichloracetic acid. For convenience, later determinations of non-protein nitrogen were made by the Sorensen method. In this case the filtrate was obtained after 8 ml. of 10% acetic acid was added to the 40 ml. of serum dilution, which was then made up to 100 ml. with saturated picric acid solution. This method gave values approximately one-third of those by the Kjeldahl method.

IV. RESULTS.

(1) Manufacture of Unwashed Butter.

The results illustrated the reliance which is placed in Queensland on the chilling effect of the wash water in hardening the butter granules so as to enable them to withstand the working process.

Table 1 shows that the wash water was normally used at a temperature averaging 8·9°F. lower than the churning temperature of the cream. The use of wash water of such low temperature was associated with granules

ranging from the size of wheat to that of a pea. These small granules of butter would permit more effective penetration of the cold and consequent even hardening of the mass of fat.

Table 1.

RELATIONSHIP OF WASH WATER TEMPERATURE AND CHURNING TEMP.

Factory.	No. of Churn Pairs.	Wash Water Temperature Range (°F).	Churning Temperature.	
			Wash Water Range (°F).	Temperature Average Difference (°F).
A	24	36–41	5–12	9
B	20	38–40	4–12	8
C	6	34–40	7–19	12
D	1	36	..	6
	51	34–41	4–19	8·9

When the manufacture of unwashed butter was attempted, the lack of the firming effect of the cold wash water was very noticeable, as the butter became soft early in the working process and reached the stage where further working was not possible before the moisture and salt were thoroughly distributed. This resulted in defects of texture and condition in a number of churns of the unwashed butter. It was found, however, that a reduction in temperature for the second half of the vat of cream by about 3–5°F. and holding the cream at that temperature for the period occupied by the first churning helped to produce a butter sufficiently firm to stand thorough working. However, even this butter was softer on removal from the churn and often had to be allowed to firm for a period prior to packing. A few checks of the temperature of the unwashed butter after completion of the manufacturing process showed it to be usually 3°F. higher than the washed control in spite of the cream having been churned at a temperature 4–5°F. lower. This higher temperature was associated with softer butter.

Table 2 sets out some details of the manufacturing methods which were associated with either good to fairly good texture or poor texture in the unwashed butter. It will be seen that, with the short holding time at the lower temperature for the unwashed churning, the cream churning temperature was, on the average, 3·6°F. lower for the butter with satisfactory texture but only 2·2°F. lower where the texture was poor. This difference was associated with a greater difference in the churning times between the washed and unwashed butters—12 minutes compared with only three minutes. Timing of the washing operations showed that the average of 15 minutes for the butter of satisfactory texture was much the same as the longer churning time associated with the lower churning temperature which proved necessary. Hence, there was no significant saving in time through omitting the washing process. There was, of course, a saving in actual labour and of the refrigeration necessary to reduce the wash water to the temperature of use.

Table 2.

MANUFACTURING DATA IN RELATION TO BUTTER TEXTURE.

Factory.	No. of Churn Pairs.	Difference in Churning Temperature (W − UW) (°F.).		Churning Time (min.).				Time for Washing. (min.).		Time Gained Through Not Washing (min.).		Moisture Droplet Count (Thousand per c.c.).			
				Unwashed.		Washed.						Washed.		Unwashed.	
		Range.	Average.	Range.	Average.	Range.	Average.	Range.	Average.	Range.	Average.	Range.	Average.	Range.	Average.
UNWASHED BUTTER OF GOOD TO FAIRLY GOOD TEXTURE.															
A ..	24	2–5	3·75	34–54	44	21–37	30	13–17	14	−11 to 9	0·5	0–31	12	0–54	16
B ..	5	0–3	1·8	36–60	43	30–60	39	17–24	20	9–20	16	14–32	25	16–28	23
C ..	3	5–7	5·7	40–50	45	23–40	33	19–20	19	− 1 to 9	4	20–68	39	28–54	41
D ..	1	..	4	..	59	..	45	..	14	..	0	..	0	..	72
	33	0–7	3·6	34–60	44	21·60	32	13–24	15	−11 to 20	3	0–68	16	0–72	26
UNWASHED BUTTER OF POOR TEXTURE.															
B ..	15	0–4	1·7	30–55	41	30–50	39	15–25	19	− 5 to 40	16	6–49	23	17–183	100
C ..	3	2–7	4·7	20–40	27	20–30	25	18–21	19	3–27	17	33–80	46	72–100	90
	18	0–7	2·2	20–55	39	20–50	36	15–25	19	− 5 to 40	16	6–80	24	17–183	99

Table 2 also illustrates the tendency for the unwashed butter to have a higher count of moisture droplets more than 30μ in diameter. This was, of course, particularly noticeable in the churnings showing poor texture, where the high counts show clearly the difficulty of obtaining thorough moisture distribution in butter which was too soft to stand prolonged working.

Lebsanft (1953) reported similar difficulties with unwashed butter during experiments in the Port Curtis district of Queensland. However, the difficulties were overcome with retention of most of the economic advantages by using buttermilk to "wash" the butter granules. The system used involved pumping the buttermilk from the first churn of the day to a vat, where it is reduced to the normal wash-water temperature prior to returning it to the churn for "washing" the granules. The process is repeated using the same buttermilk for subsequent churnings. This method overcome the manufacturing difficulties, saved refrigeration equivalent to the difference in cooling water from, say, 80°F. to 36°F. and buttermilk from, say, 44°F. to 36°F. and still gave the increased curd content expected with unwashed butter.

(2) Grading Results.

(a) Cold Storage.

The results of grading after cold storage for two and four months again showed the influence of poor texture. In such cases, as illustrated in Table 3, there was a tendency for inferior keeping quality in the unwashed butter. In the case of the unwashed butter of good to fairly good texture, however, there was no significant difference in the keeping quality of the unwashed butter and the washed controls.

Table 3.

AVERAGE FLAVOUR SCORES OF COLD-STORED BUTTER.

Factory.	No. of Churn Pairs.	Fresh.		2 Months.		4 Months.		Difference Between Washed and Unwashed Butter.		
		W.	UW.	W.	UW.	W.	UW.	Fresh.	2 Months.	4 Months.
		UNWASHED BUTTER OF GOOD TO FAIRLY GOOD TEXTURE.								
A ..	24	43·0	42·96	42·85	42·83	42·46	42·39	0·04	0·02	0·07
B ..	5	42·6	42·6	42·4	42·5	42·3	42·2	0	−0·10	0·10
C ..	3	42·17	42·0	41·5	41·17	41·25	41·25	0·17	0·33	0
D ..	1	43	43	43	42·5	42	41	0	0·5	1·0
	33	42·86	42·82	42·66	42·62	42·31	42·22	0·04	0·04	0·09
		UNWASHED BUTTER OF POOR TEXTURE.								
B ..	15	42·80	42·67	41·90	41·57	41·50	41·0	0·13	0·33	0·50
C ..	3	42·33	42·33	41·5	39·5	41	40·5	0	2·0	0·50
	18	42·72	42·61	41·86	41·23	41·42	40·92	0·11	0·43	0·50

This result as it applies to good-textured butter is in agreement with the findings of most investigators. It is possible that the difference in keeping quality found in the first investigation by Pont might have been due to some difference in the texture of the butter.

(b) Hand-cut Pats.

In the case of the pats cut by hand from 43 pairs of churnings, the washed butter developed obvious bacteriological defects in 11 instances. This was to be expected in view of the poor moisture distribution in the butter so affected. The results of gradings at two and four weeks on the remainder of the paired churnings are given in Table 4.

Table 4.

FLAVOUR SCORES OF HAND-CUT PATS AT 45–50 °F.

Averages of All Churnings except those showing Obvious Bacteriological Defects.

No. of Samples.	Fresh.		After 2 Weeks.		After 4 Weeks.		Difference between Washed and Unwashed.		
	W.	UW.	W.	UW.	W.	UW.	Fresh.	2 Weeks.	4 Weeks.
32	42·80	42·72	42·03	41·85	41·11	40·98	0·08	0·18	0·13

The results show only a very slight inferiority in the keeping quality of hand-cut pats of unwashed butter as compared with their washed controls provided the moisture distribution was sufficiently fine to prevent the occurrence of bacteriological defects. It is probable that the slight inferiority is not significant.

(c) Vacuum Treated Pats.

As discussed above, it was originally arranged for the Butter Marketing Board to accept the bulk of the churnings for use in their normal trade, thereby ensuring a convenient supply of pats for storage purposes. The process involves the re-working of the butter under vacuum in Abel machines so as to remove most of the air, improve spreadability and plasticise the butter prior to mechanical patting. This was carried out for the first four pairs of churnings from two factories. However, in three of the four cases a pronounced defect described as a "condensed-oxidised" flavour developed in the pats of unwashed butter but not in the washed controls. The defect resulted in grading penalties of 3–4 points for flavour after storage for four weeks at 45–50°F. and, in one case, a 3-point difference after two weeks. In view of the serious commercial implications of these defects, the treatment of the whole churning had to be abandoned. However, arrangements were made to treat three boxes of each of the last 22 pairs of churnings in the Abel vacuum machines and follow by machine patting.

In five of these 22 pairs a similar defect occurred which resulted in the unwashed pats receiving 2–5 points less for flavour than the controls. Thus the defect appeared in eight cases out of 26 (31%) and in butter from three of the four factories co-operating in the experiments.

In view of the description of the flavour as "condensed-oxidised," peroxide values were obtained on the pats at various ages for the last 22 pairs of churnings. Table 5 shows the peroxide values of the vacuum pats and hand-cut pats from the five churnings where the vacuum patted unwashed butter developed the defect.

Table 5.

COMPARISON OF PEROXIDE VALUES FROM VACUUM AND HAND-CUT PATS OF CHURNINGS WHERE TYPICAL FLAVOUR DEFECT OCCURRED.

Factory.	Serial No.	Type of Butter.	Peroxide Values.					
			Vacuum Pats.			Hand-Cut Pats.		
			Fresh.	2 Weeks.	4 Weeks.	Fresh.	2 Weeks.	4 Weeks.
A	16	W	·00	·00	·11	..	·05	·14
		UW	·00	·00	·00	..	·06	·09
	17	W	·02	·12	·39	..	·00	·09
		UW	·04	·00	·02	..	·02	·09
	22	W	·48	·57	·63	..	·12	·50
		UW	·04	·11	·03	..	·02	·28
B	17	W	·00	·00	·04	..	·04	·01
		UW	·00	·00	·00	..	·04	·02
	22	W	..	..	..	·13	..	..
		UW	..	..	..	·09	..	..

On the evidence in Table 5 it seems definite that the flavour was not associated with oxidation of the fat. Only in sample A22 were the peroxide values high and associated with typical oxidation defects on grading, but this occurred in the washed butter only. Apparently the antioxidant effect of the extra curd, possibly combined with some reducing effect from a slightly greater bacterial population, had prevented oxidation in the fat of the corresponding unwashed butter. The "condensed-oxidised" defect was still quite marked. The bacteriological results on these pats are outlined in Table 6, together with moisture droplet counts.

The results show little significant difference in the counts at any stage between the vacuum and hand-cut pats except possibly in the case of sample A17, where the counts increased greatly in the unwashed vacuum pats. Many of the counts are high, as was to be expected with the poor moisture distribution frequently seen and with the opportunity for bacterial multiplication given by the unavoidable delays which occurred between the time the butter was manufactured and the time it was examined. However, there appears to be no evidence that the occurrence of the defect was associated with multiplication of those bacteria which were countable by the technique employed.

It was possible, of course, that the defect was caused by the action of bacteria which were not isolated by the usual technique. In an effort to check this, analyses were made by the Kjeldahl method to determine a non-protein-nitrogen/total nitrogen ratio in the washed and unwashed vacuum

Table 6.

BACTERIOLOGICAL COMPARISON OF VACUUM AND HAND-CUT PATS SHOWING TYPICAL FLAVOUR DEFECT.

Factory.	Serial No.	Type of Butter.	Plate Counts (Thousands).						Droplet Counts (Thousands).	
			Vacuum Pats.			Hand-Cut Pats.			Vacuum Pats.	Hand-cut Pats.
			Fresh.	2 Weeks.	4 Weeks.	Fresh.	2 Weeks.	4 Weeks.		
A ..	16	W	210	560	110	150	400	290	53	14
		UW	560	1,350	100	180	370	540	88	11
	17	W	270	360	550	220	240	440	21	25
		UW	740	2,600	5,000	350	1,100	680	56	40
	22	W	240	850	700	570	1,100	430	130	18
		UW	900	1,030	1,300	260	880	850	120	26
B ..	17	W	110	160	340	125	100	150	91	26
		UW	140	140	170	130	70	460	74	98
	22	W	600	440	630	220	550	420	5	43
		UW	4,300	2,500	5,800	450	4,000	3,600	36	180

patted butter of sample A17. The results for the washed samples at two and four weeks respectively were 5·0 and 5·1, expressing non-protein nitrogen as a percentage of the total nitrogen. For the unwashed butter the respective percentages were 5·7 and 6·2. These figures did initially suggest that bacterial action might have been the cause, but, as shown below, the figures were quite normal for samples free from the defect. Therefore no explanation can be offered for the occurrence of the defect in eight of the 26 pairs of churnings examined.

(3) Chemical Results.

(a) Curd Percentages of Unwashed Butter.

One of the chief advantages claimed for the omission of washing is the resulting increase in the curd percentage of the butter. This means that more butter can be made from a given quantity of cream, with consequent economic advantages.

Table 7 shows the change in curd percentage due to the omission of washing.

Table 7.

CHANGE IN CURD PERCENTAGE DUE TO NOT WASHING.

Factory.	No. of Samples.	Washed Butter.		Unwashed Butter.		Average Increase (%).
		Range of Values (%).	Average (%).	Range of Values (%).	Average (%).	
A ..	24	0·7 – 1·1	0·90	1·1 – 1·6	1·35	0·45
B ..	20	0·5 – 1·1	0·86	1·0 – 1·5	1·27	0·41
C ..	6	0·6 – 0·9	0·71	1·1 – 1·4	1·17	0·46
D ..	1	..	0·9	..	1·3	0·4
	51	0·5 – 1·1	0·86	1·0 – 1·6	1·29	0·43

The average increase of 0·43% corresponds very closely with the results obtained by other investigators.

(b) Effect on Oxidation.

Pont (1948) showed that, in spite of a higher copper content generally, the fat of unwashed butter tended to oxidise less due to an antioxidant effect from the increased curd content. Exceptions occurred if the copper content of the butter became too high. Table 8 compares the results of peroxide values on pats at various ages after storage at 45–50°F.

Table 8.

Peroxide Values of Pats at Various Ages.

Age of Samples (Days).	No. of Paired Samples.	Average Peroxide Value.	
		Washed.	Unwashed.
0–15	13	0·06	0·01
16–30	32	0·14	0·08
31–45	34	0·29	0·19
46–60	11	0·53	0·32
	90	0·23	0·14

In only 11 cases of the 90 examinations of pairs of pats was the peroxide value higher for the unwashed butter and in most of these cases the difference was slight. Bacterial multiplication can also give an antioxidant effect, and because of a greater tendency to poor moisture distribution in the unwashed butter, this factor might have operated in some cases to increase the differences shown above. Examination of the bacteriological results suggests that this might have occurred in about 10 of the 90 cases. It is considered, nevertheless, that the results summarized above still show an overall antioxidant tendency due to the presence of the extra curd in unwashed butter.

(c) Non-protein-Nitrogen/Total Nitrogen Ratios.

As discussed above, some estimations of non-protein nitrogen as a percentage of the total nitrogen were made on the vacuum pats in an effort to detect bacterial action on the proteins. It proved necessary to substitute a Sorensen determination for non-protein nitrogen for the Kjeldahl method. The results obtained are given in Table 9.

As judged on plate counts at 30°C. on milk agar, there was generally little difference in the bacterial numbers in the washed and unwashed butters for the samples in Table 9. Hence, it seems possible that the overall tendency for higher non-protein nitrogen percentages in the unwashed butter is a characteristic of this type of butter in Queensland. McDowall (1953) in New Zealand found no increase in the non-protein nitrogen in unwashed butter and obtained results by the Kjeldahl method which were somewhat lower than those recorded here. There were slight differences in the analytical methods used;

Table 9.

ESTIMATES OF NON-PROTEIN NITROGEN AS PERCENTAGE OF TOTAL NITROGEN.

Factory.	Serial No.	Type of Butter.	Non-Protein Nitrogen as Percentage of Total Nitrogen.					
			Fresh.		2 Weeks.		4 Weeks.	
			K–K.	S–K.	K–K.	S–K.	K–K.	S–K.
A	17	W	..	..	5·0	..	5·1	1·4
		UW	..	..	5·7	..	6·2	1·8
	18	W	..	1·4	..	1·8	..	1·9
		UW	..	1·9	..	1·8	..	2·1
B	20	W	4·9	1·3	..	1·5	..	1·6
		UW	5·6	1·9	..	2·0	..	2·3
C	4	W	..	1·5	..	1·9	..	1·9
		UW	..	2·0	..	2·4	..	2·3
	5	W	..	..	..	..	..	1·7
		UW	..	..	..	..	..	2·0

(K–K both determinations by Kjeldahl method.; S–K Sorenson–Kjeldahl ratio.)

this may explain the discrepancy, but it seems equally possible that the nature of the serum may be involved. In Queensland's hotter climate it seems possible that more protein breakdown could have occurred in the original cream and that washing with water might remove a greater proportion of the non-protein nitrogen than of proteins.

(d) pH Results.

Pont (1948) showed that the pH values (presumably as measured by a glass electrode) were generally lower for the unwashed butter by about 0·1 of a unit. The pH estimations obtained in these experiments were performed by a simpler method, using a quinhydrone electrode as a check to see that each pair of churnings was of a suitable pH for good keeping quality. No significant difference between the two classes of butter was observed by this method. The butter produced for the experiments had a mean pH of 7·2, with a range from 6·6–8·0.

(4) Bacteriological Results.

A full range of bacteriological results was obtained on the samples at various stages, mainly in an effort to detect the development of bacteriological defects. Pont (1948) showed that the omission of washing tended to increase the numbers of bacteria in the finished butter. Such a conclusion could be expected on theoretical grounds. The results obtained in these experiments, however, showed no definite trend, probably because of the opportunities for bacterial multiplication which occurred between the times of manufacture and examination. The degree of bacterial multiplication depends on the fineness of distribution of the water phase in the butter, and as shown above there was considerable variation in this factor. There was often an unavoidable lag of several days between manufacture and sampling, due to delays in

forwarding butter from the factories or in its subsequent treatment in Brisbane. Although it was stored at 40°–60°F. during this period, there was obviously a variable opportunity for bacterial multiplication.

CONCLUSIONS.

The experiments confirm the findings of other investigators that it is possible to omit washing with water from the process of butter manufacture without impairing the keeping quality of bulk butter in cold storage or of patted butter held at 45–50°F. It is also shown that an appreciable economic benefit can be achieved because of the increased amount of butter which can be made from a given quantity of cream. The saving in refrigeration is also of economic importance. A further advantage demonstrated was the antioxidant effect of the extra curd.

On the other hand, it was demonstrated that under Queensland conditions it is necessary to lower the churning temperature sufficiently to prolong the churning time to about 45 minutes so as to help overcome the prevalence of defects of texture and condition. This largely counteracted any saving in time from omitting the washing process. The method of Lebsanft (1953), which involves "washing" with chilled buttermilk, overcomes this difficulty but could accentuate any deterioration in the bacteriological quality of the butter unless every care was taken.

It was shown that, unless very careful control of churning temperatures or "washing" with buttermilk was practised, inferior moisture distribution could be expected. Under such conditions the keeping quality of unwashed butter in both cold storage and at 45–50°F. would probably be impaired.

The occurrence of a typical defect in a number of cases when the unwashed butter was treated in an Abel vacuum machine prior to machine patting remains unexplained. This defect was sufficiently serious to cause concern, as a large proportion of the butter produced in Queensland is ultimately treated by such methods.

In view of the above it is felt that no recommendation should be made at present for the omission of washing as a regular practice in Queensland. However, the process could be very usefully applied in cases where difficulties were met in relation to water supplies or refrigeration.

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REFERENCES.

ARNOTT, D. R. L., GREENE, V. W., and GIBSON, D. L. 1952. Canad. Dairy Ice Cr. J. 31: 31.

DANISH STATE DAIRY EXPERIMENT STATION. 1939. Beretn. Forsoksm., Kbh. 1938-39. (Abs. in Dairy Sci. Abs. 2: 19).

JENSEN, T. M. 1942. J. Dep. Agric. Vict. 40: 528.

KOHMAN, E. F. 1919. J. Industr. Engng. Chem. 11: 36.

LEBSANFT, A. 1953. Butterfat & Solids 12: 149.

LOFTUS HILLS, G., and THIEL, C. C. 1946. J. Dairy Res. 14: 340.

MCDOWALL, F. H., SINGLETON, J. A., and O'DEA, J. J. 1953. N.Z. J. Sci. Tech. A 35: 175.

MULLER, L. L. 1952. Aust. J. Dairy Tech. 7: 44.

MULLER, L. L., and NICHOLS, L. E. 1950. Aust. J. Dairy Tech. 5: 7.

NICHOLS, L. E. 1952. Butterfat & Solids 11: 54.

PONT, E. G. 1946. J. Coun. Sci. Industr. Res. Aust. 19: 432.

PONT, E. G. 1948. J. Coun. Sci. Industr. Res. Aust. 21: 319.

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THE SEROLOGICAL EXAMINATION OF A STRAIN OF *TRICHOMONAS FOETUS* ISOLATED IN QUEENSLAND

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SUMMARY.

A strain of Trichomonas foetus **isolated in southern Queensland was compared with the two known serological types, Belfast and Manley, by the agglutination test and was found to be serologically similar to the Belfast type.**

INTRODUCTION.

The Belfast strain of *Trichomonas foetus* was first isolated by Kerr and Robertson (1941) in Northern Ireland and was found by Pierce (1949) to be well distributed in Wales and south and central England. The Manley strain of *T. foetus*, first isolated by Mahmoud (1944) from an outbreak at Manley in Cheshire, England, was confined to Wales and the central-west of England (Pierce 1949). These are the only known serological types.

Bovine trichomoniasis was first diagnosed in Queensland in 1950 (Sutherland, Simmons and Bell 1953). Since then the disease has been found in four other herds.

The serological comparison of a Queensland strain with the Manley and Belfast strains is recorded in this paper.

METHODS.

Strains.

The Belfast and Manley strains were kindly supplied by Dr. A. E. Pierce, Institute of Animal Physiology, Cambridge.

The Queensland strain, F228, was isolated from an experimental heifer that had been served 11 days previously by an infected bull. Vaginal mucus from this heifer was inoculated into modified Plastridge's medium (Sutherland *et al.* 1953) and the strain was maintained by subculturing twice weekly.

Media.

Three different media were used—modified Plastridge's medium, Douglas broth, and glucose broth on inspissated horse serum slope (see Appendix). In the last-mentioned medium, 2·5% glucose was incorporated for growth of the Belfast strain, whereas 1·5% glucose was found more suitable for cultivating the Manley strain.

Preparation of Antisera.

The suspensions for inoculation were prepared as follows:—

Belfast and Manley antigens were prepared from cultures in glucose broth on horse serum slopes. After incubation at 32°C. for 2–3 days the growth in glucose broth was harvested, centrifuged and washed. The suspension of these organisms in sterile normal saline was used as the inoculum.

Strain F228 grew well only in modified Plastridge's medium. As the agglutination was to be done with antigen containing traces of bovine serum, it was considered undesirable to inoculate rabbits with organisms in this medium, as antibodies to bovine serum would be produced. Bovine serum was therefore eliminated by passage through mice as follows: 2 ml. of a 7-day culture in modified Plastridge's medium was injected intraperitoneally into a mouse. Three days after inoculation, the mouse was killed, the peritoneal fluid harvested and diluted with three parts of saline, and this suspension inoculated into four more mice. The combined harvest of peritoneal fluid from these mice was further passaged into 12 mice. The pooled peritoneal fluid from these mice was centrifuged, and the deposit of organisms washed and re-suspended in saline to use as the inoculum.

Antiserum against each of the three strains was prepared in rabbits. Six intravenous injections were given over a period of 18 days, commencing with 0·5 ml. and increasing by 0·5 ml. to 2·5 ml. for each of the final two injections. The first injection was made with a living suspension. For subsequent injections the suspension was preserved with 0·5% formalin and kept at 4°C.

The rabbits were bled three times at intervals of two days commencing two days after the last injection, and the three lots of serum from each rabbit were pooled.

Agglutination Test.

This test was done by the method of Pierce (1947), using the readings for the different degrees of agglutination that were shown in his photomicrographs. This system of reading was also used by Kerr and Robertson (1941). The reactions range from a preagglutination zone characterised by immobilisation, which is represented by +++++, through decreasing degrees down to slight agglutination, represented by (+).

To obtain well-grown cultures for the test, the Belfast strain was grown in Douglas medium, the Manley strain in 1·5% glucose broth on an inspissated horse serum slope and strain F228 in Douglas medium inoculated with growth from modified Plastridge's medium. These antigens were tested against antisera in double dilutions ranging from 1/24 to 1/3072. All the tests were done in the one day by the one person in order to eliminate inconsistencies in interpretation of results.

RESULTS.

The results are presented in Tables 1, 2 and 3. Belfast, Manley and F228 antigens were agglutinated by their homologous antisera to high titres, but gave low titres or no agglutination with normal rabbit serum.

The F228 suspension was agglutinated to a high titre by Belfast antiserum but only to a low titre by Manley antiserum (Table 1).

A slight cross reaction occurred between Manley antigen and Belfast antiserium. Manley antigen was not agglutinated by F228 antiserum (Table 2).

Table 1.

AGGLUTINATION OF F 228 ANTIGEN BY SERA PREPARED AGAINST BELFAST, MANLEY, AND F228 STRAINS OF *T. foetus*.

Antiserum.	Dilution of Serum.							
	1/24	1/48	1/96	1/192	1/384	1/768	1/1536	1/3072
F228	++++	+++	+++	++(+)	+	(+)	(+)	—
Belfast	+++	++(+)	+(+)	+	+	(+)	—	—
Manley	+	(+)	—	—	—	—	—	—
Control	—	—	—	—	—	—	—	—

Table 2.

AGGLUTINATION OF MANLEY ANTIGEN BY SERA PREPARED AGAINST BELFAST, MANLEY, AND F228 STRAINS OF *T. foetus*.

Antiserum.	Dilution of Serum.							
	1/24	1/48	1/96	1/192	1/384	1/768	1/1536	1/3072
F228	—	—	—	—	—	—	—	—
Belfast	+ (+)	+	(+)	—	—	—	—	—
Manley	++++	++(+)	++	++	+	(+)	—	—
Control	—	—	—	—	—	—	—	—

Table 3.

AGGLUTINATION OF BELFAST ANTIGEN BY SERA PREPARED AGAINST BELFAST, MANLEY, AND F228 STRAINS OF *T. foetus*.

Antiserum.	Dilutions of Serum.							
	1/24	1/48	1/96	1/192	1/384	1/768	1/1536	1/3072
F228	+++	++	++	++	+(+)	+	(+)	—
Belfast ..	++++	+++(+)	++(+)	++(+)	++(+)	++	+(+)	(+)
Manley ..	+(+)	+	+	+	(+)	—	—	—
Control ..	(+)	(+)	—	—	—	—	—	—

Belfast antigen was agglutinated by F228 antiserum to a high titre but gave only a slight cross reaction with Manley antiserum (Table 3).

DISCUSSION.

The results show that the strain of *T. foetus* isolated in Queensland is antigenically similar to the Belfast strain.

Four of the five herds in which trichomoniasis has been diagnosed in Queensland exchanged cattle directly and indirectly with the herd from which strain F228 was isolated. It is reasonable to assume that the infections in

these four herds were due to one antigenic type. The strain isolated from the fifth herd died before typing could be done. Until more strains have been isolated and typed it would be wise to use both Manley and Belfast types in the agglutination test for diagnosis of trichomoniasis in Queensland.

REFERENCES.

KERR, W., and ROBERTSON, M. 1941. An investigation into the infection of cows with *Trichomonas foetus* by means of the agglutination reaction. Brit. Vet. J. 97: 351-65.

MAHMOUD, A. 1944. Isolation of *Trichomonas foetus* (Riedmüller, 1928) in bacteria-free culture by the use of penicillin. Ann. Trop. Med. Paras. 38: 219-22.

PIERCE, A. E. 1947. The serum agglutination reaction of *Trichomonas foetus*, Riedmüller, 1928. Lab. J. 8: 238-44.

———————. 1949. The agglutination reaction of bovine serum in the diagnosis of trichomoniasis. Brit. Vet. J. 105: 286-94.

SUTHERLAND, A. K., SIMMONS, G. C., and BELL, A. T. 1953. An outbreak of bovine trichomoniasis in Queensland. Aust. Vet. J. 29: 67-9.

APPENDIX.

Preparation of Glucose Broth on Inspissated Horse Serum.

Nutrient broth was prepared as follows:—

60 gm. Difco meat extract, 60 gm. sodium chloride and 120 gm. Bacto peptone were dissolved by heating in 12 litres of distilled water. The pH was adjusted to 7·2 with 1N sodium hydroxide, and 0·0125% calcium chloride was then added. 1·5% glucose was added to the medium for the Manley strain and 2·5% to the medium for the Belfast strain. The pH was adjusted to 7·2–7·4 and the broth autoclaved at 15 lb. for 15 minutes.

The broth was dispensed in 8 ml. quantities into 1 oz. McCartney bottles containing an inspissated horse serum slope. The slope was prepared by pipetting 5 ml. horse serum into the bottle and inspissating for one hour at 80–85°C. on each of three consecutive days. Paraffin oil to a depth of ¼ inch was layered onto the surface of the broth, the medium steamed for 20 minutes and then autoclaved at 15 lb. for 15 minutes.

Preparation of Douglas Medium.

The formula was obtained from the Animal Research Station, Wallaceville, New Zealand.

The medium consists of a digest broth covering an inspissated bovine serum slope.

The broth was prepared as follows:—

Ox heart, free from fat, was minced and mixed with tap water in proportion of 1 lb. meat to 1 litre water. The mixture was stirred and pH adjusted to 8·5 with 20% potassium hydroxide. After heating to 75°C., the mixture was cooled to 50°C., then, 1% Allen and Hanbury's Liquor Trypsin Co. added. The pH was restored to 8·5 with 20% potassium hydroxide, and digestion allowed for 1½ hours keeping the pH constant. At the end of this period a further 1% Liquor Trypsin Co. was added, and digestion continued for a further 1½ hours. The pH was adjusted to 5·5 with concentrated

hydrochloric acid, and the digest heated gently until boiling. The hot digest was filtered through gauze and filter paper, and to the filtrate was added 0·25% sodium chloride and 0·0125% calcium chloride. When dissolved, the pH was adjusted to 8·2 with 20% potassium hydroxide. After cooling, the digest was filtered through filter paper, 2·5% glucose added and pH adjusted to 7·2–7·4 with normal hydrochloric acid.

The slope was prepared by pipetting 5 ml. bovine serum into a 1 oz. McCartney bottle and inspissating at 80–85°C. for one hour on each of three consecutive days.

To prepare the medium, 10 ml. of broth was added to each bottle containing the slope. Liquid paraffin to a depth of ¼ inch was layered onto the surface of the broth. The medium was steamed for 20 minutes, then autoclaved at 15 lb. per square inch for 15 minutes. The pressure was reduced gradually to prevent violent boiling of the fluid portion of the medium.

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